

REMARKS

Summary of the Office Action

In the Office Action of April 21, 2008, the Examiner acknowledged Applicant's previous election of claims 31-46 and 53-60. Additionally, the Examiner objected to claims 31-46 and 53-60 for various informalities. Further, the Examiner rejected claims 31-46 and 53-60 as being indefinite, for omitting essential elements, and for failing to comply with the enablement requirement.

In regard to the substantive examination of the claims, the Examiner rejected claims 31-41 as being anticipated by U.S. Patent No. 6,284,236 issued to Wiley (hereinafter referred to as "Wiley"). The Examiner also rejected claims 31-46 and 53-60 as being anticipated by an article by Aiuti et al. entitled "Hematopoietic Support and Cytokine Expression of Murine-Stable Hepatocyte Cell Lines (MMH) (hereinafter referred to as "Aiuti"). Additionally, the Examiner rejected claims 31-39 and 42-46 as being anticipated by U.S. Patent No. 5,521,076 issued to Mulligan (hereinafter referred to as "Mulligan") as evidenced by a Becton-Dickinson website (hereinafter referred to as "BD"). Finally, the Examiner rejected claims 31-46 and 53-60 as being unpatentable obvious over Aiuti in view of U.S. Patent No. 7,118,746 issued to Naughton (hereinafter referred to as "Naughton").

Summary of the Amendments

Upon entry of the present Response to Office Action, Claims 31, 34-38, 40, 42-44, and 53-60 will have been amended. Additionally, Claims 32, 33, 39, 41, and 45-52 will have been cancelled. As such, Claims 31, 34-38, 40, 42-44, and 53-60 remain currently pending. By the present amendment, Applicant submits that the rejections have been overcome and respectfully requests reconsideration of the outstanding Office Action.

Applicant's Response

1. Objection to Claims 31-46 and 53-60

Applicant respectfully submits that all of the Examiner's objections to the claims have been overcome by the present amendments. In particular, all of the claims have been amended to include the appropriate introductory article of either "A" or "The"; the words "characterized for" have been removed from claim 37; the words "the" and "medium" have been appropriately inserted into claim 40; certain words have been modified to the present participle form in claim 53; and a period has been inserted at the end of claim 60.

Accordingly Applicant requests that the Examiner withdraw all of the objections to the claims pursuant to the present amendment.

2. Section 112, Second Paragraph, Rejection of Claims 31-46 and 53-60

Applicant respectfully submits that the present amendment to the claims has overcome the indefiniteness rejections.

In particular, claim 31 has been amended to remove the language "conditioned by cytokines and soluble factors released by immortalized untransformed hepatocytes that are differentiated, polarized epithelial cells." Claim 31 is now directed toward a culture medium "conditioned by MMH cells (c-Met Transgenic Murine Hepatocyte)" which is fully consistent with the specification.

In regard to claims 37-39, claim 31 no longer includes the language of conditioning with a cytokine, and, as such, there is no longer any ambiguity as to whether the cytokines in claims 37-39 are the same as the cytokines in claim 31. Additionally, claim 39 has been cancelled by this amendment.

In regard to claims 42-46, the first instance of a "culture medium" discussed in claim 42 has been amended to recite "the conditioned culture medium according to claim 1" as opposed to the second instance which remains as a "culture medium." As such, the two uses are clearly distinguished in the currently amended claim in that the second occurrence discusses incubating MMH cells in a culture medium in order to obtain the *conditioned* culture medium of claim 1. Furthermore, the use of "culture medium" in claim 44 is now clear in that it is not directed

toward the *conditioned* culture medium, but rather the starting culture medium. Furthermore, the tradenames present in claim 44 have been capitalized and the TM symbol has been inserted where appropriate. Applicant respectfully submits that no other common name can be used to describe these culture mediums.

Finally, in regard to claim 60, "laboratory means" has further been amended to include "for cell culture" which is known to a person having ordinary skill in the art, and may include sterility means, gloves, plastic-ware, etc.

In regard to the rejection of claims 31-46 and 53-60 as being incomplete for omitting essential elements, Applicant respectfully submits that, with the present amendments, all essential elements are present in the claims. In particular, claim 31, as currently amended, now discloses all of the required chemical components, i.e., a culture medium conditioned by MMH cells, free from conditioning cells. The present language no longer describes the culture medium functionally, but provides the exact requirements of the culture medium. Furthermore, the language, "when used" is no longer present and the claims are believed to now be definite. Applicant respectfully submits that "free from conditioning cells" is not indefinite, but, rather, would be understood by a person having ordinary skill in the art to mean that the conditioning cells have been separated from the culture medium.

Additionally, in regard to claim 38, Applicant respectfully submits that this claim is definite because the term "depleted" indicates that some amount of the biological molecule has been removed. This claim thus requires that some of the intended biological material has been removed, and thus a person having ordinary skill in the art would understand the scope of the invention.

In regard to claim 42, Applicant submits that the current amendment has rendered this claim definite. In particular, the claim now states that the hepatocytes are separated from "the conditioned culture medium" and the language "before the use" has been removed. As such, Applicant submits that this claim is now definite.

Regarding claims 46 and 33, these claims have been cancelled and accordingly this rejection is rendered moot. Regarding claims 34 and 35, the word "cultured" has been removed, thereby overcoming the insufficient antecedent basis rejection. Regarding, claims 31 and 53,

the term “adult” has been removed, thereby rendering the insufficient antecedent basis rejection of claims 34, 36, 54, and 56-59 moot.

Finally, in regard to claim 31, the language “when used” has been removed and instead the claim is now directed toward a culture medium for maintenance and proliferation of mammalian cells. Accordingly, Applicant respectfully submits that this indefinite rejection has been overcome.

Accordingly, Applicant respectfully submits that all of the 35 U.S.C. § 112, second paragraph, rejections have been overcome and requests that these rejections be withdrawn.

3. Section 112, First Paragraph, Rejection of Claims 31-46 and 53-60

The Examiner rejected claims 31-46 and 53-60 because the Examiner felt that it was unclear whether the biological materials are (I) currently available from repository, commercial sources, or via methods known in the art or if the said materials are (II) not so available/obtainable.

In response, Applicant respectfully submits that the biological materials are readily available via commercial sources and/or via methods known in the art. In particular, the isolation and characterization of c-Met Murine Hepatocytes has been described in several scientific reports, cited in the present Application at page 2, lines 28-32.

Applicant refers in particular to Amicone et al., EMBO J. 1997, 16: 495-503 (hereinafter referred to as “Amicone”), which describes the preparation of a cyto-Met and AT-cyto-MET transgenic mice and the isolation of primary hepatocytes from their liver (a schematic of the transgenes is given in the reference, Fig. 1A, page 496).

Isolation of primary hepatocytes is easily achieved by mechanical dissociation of the liver and cells plating on collagen coated dishes (see p. 497, par. Titled “*Transgenic hepatocytes are permissive for the establishment of cell lines*”). In particular, Applicant directs the Examiner’s attention to the sentence bridging the end of page 497 and the beginning of page 498:

One or more immortalized cell lines per embryo or newborn animal, harvested from three different transgenic lines, was obtained reproducibly. Out of 33 immortalized hepatocyte lines established, named MMH, nine have so far been kept in culture for > 12 months.

Therefore, Applicant submits that MMH cell lines are known in the art and easily available to a person having ordinary skill in the art by standardized isolation techniques from the liver of c-Met transgenic mice.

These mice are available, since their disclosure in Amicone (cited in the specification at page 2, line 28), upon request to: Prof. Marco Tripodi, Fondazione Istituto Pasteur Cenci-Bolognetti, Dipartimento di Biotecnologie Cellulari ed Ematologia, Universita La Sapienza Roma, v. le Regina Elena 324, 00161 ROMA.

4. Section 102(b) Rejection of Claims 31-41 over Wiley

The Examiner contends that Wiley anticipates the claims by teaching a cell culturing media, wherein the media was replaced with DMEM and cytokines. *Office Action, Page 13.*

Applicant's independent Claim 31 as currently amended recites, inter alia, "A culture medium conditioned by MMH cells (c-Met Transgenic Murine Hepatocyte) ..."

Applicant respectfully submits that the limitation to an MMH conditioned cell-culture medium, taken together with the definition of a "conditioned medium" in the Specification (see, e.g., page 6) removes Wiley as being an appropriate anticipatory reference.

For the foregoing reasons and because Wiley fails to disclose the above-noted features of the present invention, Applicant submits that Wiley fails to disclose each and every recited feature of the present invention as recited in independent Claim 31.

Accordingly, Applicant submits that the Examiner has failed to provide an adequate evidentiary basis to support the rejection under 35 U.S.C. § 102(b) and that the present rejection of Claim 31 is improper and should be withdrawn

Applicant further submits that the Claims 34—38 and 40 are allowable at least for the reason that these claims depend on allowable independent Claim 31 and because these claims recite additional features that further define the present invention.

5. Section 102(b) Rejection of Claims 31-46 and 53-60 over Aiuti

The Examiner contends that Aiuti teaches a medium composition comprising cytokines/soluble factors and that Aiuti teaches MMH-coculture medium and MMH-conditioned

medium. *Office Action, Page 13*. The Examiner, however, admits that Aiuti teaches that MMH-conditioned media does not support long-term maintenance. *Id. at Page 14*.

Applicant's independent Claim 31 as currently amended recites, inter alia, "A culture medium conditioned by MMH ... free from conditioning cells, for maintenance ... of mammalian cells."

Aiuti discloses, as the Examiner acknowledged, the use of MMH-conditioned medium in the presence of MMH cells (co-culturing), i.e., it describes the "co-culturing" of cells. Aiuti also states that, the cell-cell contact is necessary for long term maintenance, and the overall teaching of Aiuti is summarized in the Final Remarks (last sentence of page 1653, left column):

Taken together, our data provide evidence at the molecular and functional level for a direct role of both embryonic and newborn-derived hepatocytes in sustaining hemopoietic cell proliferation and differentiation.

This is clearly different from what is claimed in the present invention, according to amended claim 31, where a conditioned medium free of cells is claimed and where one of the advantages of the present medium is the long-term culture of stem cells (see attached Declaration).

Furthermore, experimental evidence regarding advantages in the use of the current culture medium (which according to the Specification, comprises several growth factors and/or cytokines so that exogenous cytokines may not be needed for growing certain types of cells. *Page 8, lines 6-8*.) over a medium comprising cytokines from an exogenous source have been provided. Although the term "conditioned" excludes culture medium where cytokines have only been added and proportioned by man, as previously discussed above, Applicant submits that such a comparison has been presented in the Specification, for example in Figure 1.

Figure 1 (described in the Specification on page 11, lines 7-19) shows that bone marrow-derived hemopoietic cell fold-proliferation in the presence of IMDM (a common culture medium added with 10% FBS and antibiotics, pointed bar in Fig. 1) is almost undetectable at the third day of culture and is close to zero at days 7 and 14, compared to a five-fold cell increase in the

presence of MMH-CM conditioned medium (white bar) or to a six-fold increase in co-culture (black bar) at the 14th day.

The results of this experiment show a clear advantage in the use of MMH-Conditioned Medium, either compared to an IMDM culture medium, or to the co-culture system. Applicant believes that the comparative data given in the Specification sufficiently demonstrates the advantages of the presently claimed conditioned culture medium over the prior art medium. These advantages are particularly observed with respect to the maintenance of stemness potential and proliferation, as clearly shown in the attached Declaration.

In fact, the Specification generally relates to three properties (maintenance, proliferation, and differentiation), although by “differentiation” it was intended that stem cells cultured in MMH-CM maintain their ability to *further differentiate*, once placed in a “differentiative” environment, as shown in the Declaration. The Declaration shows *in vivo* experiments on hematopoiesis and neoangiogenesis of stem cells grown for a long time in MMH-CM.

Further, results obtained with stem cells are summarized in the present Specification at Example 3 on page 13, lines 8-17, wherein CD34⁺ CD133⁺ isolated cells have been maintained (“for 3 weeks”, page 13, line 11), expanded (“> 8-fold”, page 13, line 13), and differentiated toward the endothelial cell line, upon incubation in MMH-conditioned medium for three weeks.

This aspect, maintenance of cell stemness in the short *and long term* (up to 108 days) has been confirmed by the additional assays presented in the Declaration, where it has been demonstrated that this culture medium sustains the clonogenic potential of CD34⁺ stem cells at a very early stage of stemness, as demonstrated by their ability to give rise to both the endothelial and hemopoietic precursors once placed in the correct environment.

For the foregoing reasons and because Aiuti fails to disclose the above-noted features of the present invention, Applicant submits that Aiuti fails to disclose each and every recited feature of the present invention as recited in independent claim 31.

Accordingly, Applicant submits that the Examiner has failed to provide an adequate evidentiary basis to support the rejection under 35 U.S.C. § 102(b) and that the present rejection of claim 31 is improper and should be withdrawn.

Applicant further submits that the claims 34-38 and 40 are allowable at least for the reason that these claims depend on allowable independent Claim 31 and because these claims recite additional features that further define the present invention.

Similarly, Applicant submits that claims 42-44 and 53-60 are allowable for at least the reason that they incorporate the same requirements discussed above that are not taught or suggested by the cited references.

6. **Section 102(b) Rejection of Claims 31-39 and 42-46 over Mulligan**

The Examiner contends that Mulligan teaches culturing a PRIMARIA™ plate of hepatocytes in culture media. *Office Action, Page 16.*

Applicant submits the currently amended claims are novel in light of the Mulligan reference at least for the reason that the specific passages in column 13, cited by the Examiner, clearly refer to the preparation of a culture medium where hepatocytes are cultured to produce viruses (viral stock) and not to “condition” a culture medium, as in the process of the present Application.

A person having ordinary skill in the art looking for the preparation of a cell culture medium to be used also in stem cells culture, would not turn to the Mulligan reference, as viruses and retroviruses, the preparations of which are there described, are to be absolutely avoided in a cell culture medium, as viruses and retroviruses represent **pathogens**.

On the contrary, for the preparation of the present conditioned culture medium, filtration may be carried out in order to remove conditioning cells, as described in the present Specification at page 6, lines 13 and 14, “the cells used to condition the medium are removed with standard filtration techniques. Following the removal of the conditioning cells, the medium can be used...”

As such, the currently amended claims which require conditioning the culture medium with MMH cells (c-Met Transgenic Murine Hepatocytes) are believed to overcome this rejection in view of Mulligan. Also, the Mulligan reference cannot be properly combined with the other cited references for the reasons given above, namely that viral or retroviral preparation is to be avoided in a cell culture and represents a completely different field from cell culture.

For the foregoing reasons and because Mulligan fails to disclose the above-noted features of the present invention, Applicant submits that Mulligan fails to disclose each and every recited feature of the present invention as recited in independent claim 31.

Accordingly, Applicant submits that the Examiner has failed to provide an adequate evidentiary basis to support the rejection under 35 U.S.C. § 102(b) and that the present rejection of claim 31 is improper and should be withdrawn.

Applicant further submits that the claims 34-38 are allowable at least for the reason that these claims depend on allowable independent Claim 31 and because these claims recite additional features that further define the present invention.

Similarly, Applicant submits that claims 42-44 are allowable for at least the reason that they incorporate the same requirements discussed above that are not taught or suggested by the cited references.

7. Section 103(a) Rejection of Claims 31-46 and 53-60 over Aiuti in view of Naughton

The Examiner admits that Aiuti does not teach the possible component combinations, mode of separation, or reaction times as recited in Applicant's claims. *Office Action, Page 17*. In order to overcome this deficiency, the Examiner cites to Naughton for teaching that the claimed alternatives are known for the purpose of conditioned media. *Office Action, Pages 17-18*.

In response, Applicant submits that the selection of an MMH cells conditioned medium represents a purposive and advantageous selection, particularly in view of the results obtained with *human stem cells* (isolated from cord blood as described in the Specification at page 10, lines 9-31) in terms of maintenance, expansion, and later possibility of differentiation of said stem cells toward different cell lineages (hemopoietic and endothelial, *see* page 13).

The Examiner's attention is directed toward the results obtained with stem cells, summarized in the Specification (*see* Example 3, page 13, lines 8-17) wherein CD34⁺ CD133⁺ isolated cells have been maintained "for 3 weeks", expanded "> 8-fold", and differentiated toward the endothelial cell line, upon incubation in MMH-conditioned medium for three weeks. The use of CD34⁺ cells as a stemness model able to differentiate either in hemopoietic or endothelial lineage precursors is well known.

These results, as more deeply investigated and confirmed by the additional experiments described in the attached Declaration, were not foreseen, as discussed above, by Aiuti because direct cell-cell contact was disclosed as a necessary feature for stem cell maintenance in culture.

Accordingly, Applicant submits that no proper combination of Aiuti and Naughton discloses or suggests at least the above-noted features of the present invention, and thus, the rejection of at least independent Claim 31 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

Applicant further submits that the Claims 34-38 and 40 are allowable at least for the reason that these claims depend on allowable independent Claim 31 and because these claims recite additional features that further define the present invention. Additionally, Applicant submits that claims 42-44 and 53-60 are allowable for at least the reason that they incorporate the same requirements discussed above that are not taught or suggested by the cited references.

Conclusion

Applicant respectfully submits that each and every pending claim of the present invention meets the requirements for patentability under 35 U.S.C. §§ 112, 102, and 103, and respectfully requests that the Examiner indicate allowance of each and every pending claim of the present invention.

In view of the foregoing, it is submitted that none of the references of record, either taken alone or in any proper combination thereof, anticipate or render obvious Applicant's invention as recited in each of Claims 31, 34-38, 40, 42-44, and 53-60. The references of record have been discussed and distinguished, while significant claim features of the present invention have been pointed out.

Accordingly, reconsideration of the outstanding Office Action and allowance of the present application and all the claims therein are respectfully requested and now believed to be appropriate.

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If any additional fee is required, please charge Deposit Account Number 19-4330.

Respectfully submitted,

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